EXHIBIT E

Endothelin ET_A Receptor Antagonist Blocks Cardiac Hypertrophy Provoked by Hemodynamic Overload

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Background We have recently shown that angiotensin II–induced hypertrophy of cultured rat cardiomyocytes was partially blocked by an endothelin (ET) receptor antagonist (BQ123) selective for the ET_A subtype, suggesting the possible involvement of endogenous ET-1 in the mechanism of cardiac hypertrophy in vitro. In the present study, we studied the in vivo blockade effects of BQ123 on cardiac hypertrophy provoked by left ventricular overload with aortic banding in adult rats.

Methods and Results Forty rats were divided into four groups: (1) sham-operated rats without BQ123 administration, (2) rats with aortic banding without BQ123 administration, (3) sham-operated rats with BQ123 administration, and (4) rats with aortic banding with BQ123 administration. BQ123 (250 μg/h) was administered continuously by an osmotic pump starting 24 hours before operation. BQ123 blocked increases in the ratio of left ventricular weight to body weight and in the diameter of cardiomyocytes provoked by aortic banding at 1 week, but those blockade actions were no longer observed at 2 weeks. Skeletal α-actin and atrial natriuretic peptide (ANP) mRNA in the left ventricle, transcriptional markers for cardiac hypertrophy, significantly increased in the rats with aortic banding at 1 week and 2 weeks. In the rats with BQ123 administration, despite the hemodynamic overload, skeletal α-actin and ANP mRNA in the left ventricle remained at the control levels at 1 week; however, those blockade actions were abolished at 2 weeks. Plasma ET-1 levels increased after aortic banding, peaking at 24 hours, then returned to the basal level at 4 days. Prepro-ET-1 mRNA levels in the left ventricle also increased 24 hours after aortic banding, then declined to the basal level at 4 days.

Conclusions These data suggest that endogenous ET-1 synthesized in the cardiovascular system plays a role in the mechanism of cardiac hypertrophy during the early phase of pressure overload in vivo. (*Circulation*. **1994;89:2198-2203.**)

Key Words • RNA • atrial natriuretic peptide • endothelin • aorta

Cardiac hypertrophy provoked by mechanical stress, such as hemodynamic overload, results primarily from enlargement of cardiomyocytes rather than proliferation. During the development of experimental cardiac hypertrophy induced by workload, transcripts of several muscle-specific genes usually expressed by the embryonic and the fetal ventricle, for example, β-myosin heavy chain, skeletal α-actin, and atrial natriuretic peptide (ANP),[1] can be reexpressed. However, during the development of cardiac hypertrophy induced by neurohumoral factors, such as α_1-adrenergic agonist,[2,3] angiotensin II,[4] and several growth factors,[5,6] a subset of these muscle-specific genes can also be reactivated. Therefore, it appears that cardiac hypertrophy induced by mechanical overload and that induced by neurohumoral factors share signaling pathways in common.

Endothelin-1 (ET-1) is a 21-amino-acid residue vasoconstrictor peptide originally characterized from the supernatant of cultured porcine aortic endothelial cells.[7] This endothelium-derived vasoconstrictive peptide has also been shown to be a potent growth factor in a variety of cells.[8,9] We[10] and other investigators[11] have previously demonstrated that ET-1 induces hypertrophy of cultured rat cardiomyocytes, with concomitant induction of several cardiac muscle–specific genes, such as myosin light chain 2, α-actin, and troponin I. Recently, we showed that cultured rat cardiomyocytes express abundant prepro-ET-1 (ppET-1) transcripts and release mature ET-1 into culture medium[12] and that cardiomyocyte hypertrophy stimulated by angiotensin II is partially blocked by a receptor antagonist (BQ123) selective for the ET_A subtype.[12] These in vitro results led us to speculate that endogenous ET-1 produced by cardiomyocytes may be involved in the mechanism of cardiac hypertrophy via an autocrine/paracrine pathway.

To ascertain whether endogenous ET-1 mediates cardiac hypertrophy provoked by workload in vivo, we studied the blockade effect of BQ123 on left ventricular (LV) hypertrophy and the expression of skeletal α-actin and ANP mRNA after abdominal aortic banding in adult rats and the changes of circulating ET-1 and ventricular ppET-1 mRNA levels after aortic banding.

Methods

Drugs and cDNAs

BQ123[13] was provided by Banyu Pharmaceutical Co Ltd. cDNAs for rat ppET-1,[7] rat ANP,[14,15] and human GAPDH[16] used for probes were generously provided by Dr M. Masaki (Kyoto University, Japan), Dr H. Matsuo (National Cardiovascular Center Research Institute, Osaka, Japan), and Dr K. Webster (SRI International, Menlo Park, Calif), respectively. cDNA probes for the 3′ untranslated region of rat skeletal

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α-actin (184 bp) and cardiac α-actin (129 bp) were synthesized as previously described.[6]

Experimental Animals and Surgical Procedures

Experiment 1

Forty adult Wistar-Imamichi male rats weighing approximately 270 g were divided into four groups: (1) sham-operated rats without BQ123 administration, (2) rats with aortic banding without BQ123 administration, (3) sham-operated rats with BQ123 administration, and (4) rats with aortic banding with BQ123 administration. Saline only or BQ123 dissolved in 0.9% saline was administered continuously by a subcutaneously implanted osmotic pump (model 2ML1 or 2ML2, Alza Corp) starting 24 hours before operation. Continuous infusion of BQ123 (250 μg/h) maintained constant plasma levels at approximately 0.5 μmol/L as evaluated by high-performance liquid chromatographic analysis. Twenty-four hours after implantation of osmotic pumps, aortic banding or sham operation was carried out by the method originally described by Jouannot and Hatt[17] with minor modifications. Briefly, the animals were anesthetized with sodium pentobarbital (30 mg/kg IP) and subjected to a sham operation or aortic banding at the suprarenal abdominal aorta by a blunt 22-gauge needle to establish the diameter of the ligature. The sham procedure for control rats included injection of the same amount of anesthesia, approximately the same size of incision for sham and aortic banding operations, and placement of a loosely tied ligature at the identical position at the abdominal aorta. At 1 week and 2 weeks after the operation, aortic and LV pressures were measured by a saline-filled polyethylene catheter connected to a Statham transducer (P10EZ, Gould, Inc) as previously described,[18] and then the rats were killed by exsanguination. The LV (LV free wall plus interventricular septum) free of atria and right ventricular free wall were weighed. Tissue specimens of LV free wall were immediately frozen in liquid nitrogen.

Experiment 2

For measurement of plasma ET-1 and ventricular ppET-1 mRNA levels, aortic banding and sham operation were performed in 39 rats in the same manner as in experiment 1. Six hours and 1, 2, and 4 days after operation (four or five rats per group), rats were anesthetized and blood samples were drawn from the right ventricle by cardiac puncture. After the rats were killed by exsanguination, LV free walls were excised and immediately frozen in liquid nitrogen. LV tissue specimens and blood samples from aortic-banded and sham-operated rats without BQ123 administration in experiment 1 were used for 1-week and 2-week measurements. Four rats without operation served as controls.

RNA Preparation and Analysis

Total RNA from LV free wall was isolated by guanidine thiocyanate and centrifuged through a 5.7 mol/L CsCl cushion.[19] RNA was analyzed by Northern and dot blotting methods by hybridization to ^{32}P-labeled cDNA probes prepared by the random-primer method[20] in the same conditions as previously described.[10,21] The membranes (Magnagraph nylon, Micron Separations Inc) were washed twice with 5× SSPE/10% SDS at room temperature, twice with 1× SSPE/10% SDS at 37°C for 15 minutes, and once with 0.1× SSPE/10% SDS at 65°C for ppET-1 probe or 37°C for other probes for 15 minutes. Radioactivity of dot blot analysis was quantified by an image analyzer system (BAS2000, Fuji Film Corp). Results were normalized by signals of GAPDH mRNA. Autoradiography was performed on Fuji RX film with an intensifying screen at −80°C.

Measurements of Cell Diameter

The samples, fixed in 10% formalin, were cut into 4-μm sections and stained with hematoxylin and eosin. Individual myocyte diameters were measured directly with a micrometer (Nikon) at a magnification of ×200 by an observer who was not informed about the group of samples. The LV free wall was subdivided approximately into thirds at the endomyocardium, midmyocardium, and epimyocardium, and two or three microscopic fields per region, for a total of six to eight fields, were chosen for analysis. Diameter measurements were made across the nuclei and were restricted to myocytes containing nuclei in the center of the fiber in both transversely and longitudinally cut cells. Five to 10 myocytes per field, in total 50 myocytes per animal, were analyzed.

Radioimmunoassay

Blood samples were immediately transferred to chilled siliconized disposable glass tubes on ice containing aprotinin (500 kallikrein inactivator units/mL) and EDTA (1 mg/mL). Plasma was separated by centrifugation for 10 minutes at 4°C and stored at −80°C until analysis. Plasma immunoreactive ET-1 was determined by a specific radioimmunoassay as previously described[22]; the antibody used cross-reacted fully with ET-1, 2% with big ET-1, but <0.1% with ET-2 and ET-3.[23]

Statistical Analyses

Two-way ANOVA (for experiment 1) or one-way ANOVA (for experiment 2) followed by multiple comparison methods by Scheffé's test were used for statistical analyses. A value of $P<.05$ was considered significant.

Results

Changes in Aortic Pressure, LV Weight, and Diameter of Cardiomyocytes (Experiment 1)

At 1 week and 2 weeks after operation, aortic systolic pressure was elevated in the aortic banding groups. Administration of BQ123 did not affect aortic pressure in either sham-operated or aortic banding groups at 1 week and 2 weeks (Table 1).

The LV/body weight ratio significantly ($P<.05$) increased in rats with aortic banding at 1 week and 2 weeks after operation. Administration of BQ123 blocked the increased LV/body weight ratio provoked by load at 1 week, but the blockade action was no longer observed at 2 weeks (Table 1). The diameter of cardiomyocytes in the LV, as evaluated by morphometry, also increased in rats with aortic banding at 1 and 2 weeks after operation; however, increases in the cell diameter were not observed in rats with aortic banding plus BQ123 administration at 1 week. BQ123 did not inhibit increases in the diameter of cardiomyocytes in the LV at 2 weeks after aortic banding. Both the LV/body weight ratio and the diameter of cardiomyocytes in the LV were unaffected by BQ123 in sham-operated rats during the experiment (Table 2).

Changes in LV Expression of Skeletal and Cardiac α-Actin and ANP mRNA

mRNA levels of skeletal α-actin, ie, the fetal isoform of sarcomeric actin, significantly increased in the rats with aortic banding at 1 and 2 weeks after operation, whereas cardiac α-actin mRNA levels were unchanged. ANP mRNA, which is minimally expressed in the LV of normal heart, was also increased by aortic banding at 1 and 2 weeks. In the rats with BQ123 administration, despite the hemodynamic overload, skeletal α-actin and ANP mRNA levels remained close to control levels at 1 week, whereas this blockade action was no longer observed at 2 weeks (Figs 1, 2A, and 2B).

Table 1. Aortic Systolic Pressure, Body Weight, and Left Ventricular Weight at Death in Experiment 1

Time of Death	Operation and Treatment	n	Aortic Systolic Pressure, mm Hg	Body Weight, g	LV Weight, mg	LV Weight, mg/Body Weight, g
1 wk	Sham+saline	5	125±7.4	305±5.0	704±5.0	2.31±0.02
	AOB+saline	5	164±4.9*	298±3.1	803±13.3*	2.70±0.04*
	Sham+BQ123	5	118±12.6	292±3.2	640±16.6	2.21±0.04
	AOB+BQ123	5	169±6.0*	300±10.8	664±31.8†	2.23±0.14†
2 wk	Sham+saline	5	117±8.3	344±9.3	764±16.3	2.23±0.06
	AOB+saline	5	191±15.0*	335±15.3	994±25.3*	2.83±0.13*
	Sham+BQ123	5	112±3.6	343±12.5	808±42.5	2.29±0.89
	AOB+BQ123	5	180±14.3*	348±20.6	948±20.6*	2.72±0.10*

n indicates number of rats; LV, left ventricle; and AOB, aortic banding. Values are mean±SEM.
*P<.05 compared with sham-operated group; †P<.05 compared with AOB+saline group.

Changes in Ventricular Expression of ppET-1 mRNA and Plasma ET-1 Levels

Aortic systolic pressure, body weight, LV weight, and the LV/body weight ratio during the early phase of pressure overload (experiment 2) are summarized in Table 3. Aortic systolic pressure was higher in rats with aortic banding than in sham-operated rats as early as 6 hours. Body weight was not significantly changed during the course of the experiment; however, LV weight and the LV/body weight ratio were significantly higher in rats with aortic banding compared with those in sham-operated rats at 4 days.

Changes in LV ppET-1 mRNA levels are shown in Fig 3 (representative autoradiograms of Northern blot analysis) and Fig 4 (the quantitative data of dot blot analysis). LV ppET-1 mRNA levels were low in control and sham-operated rats. After aortic banding, ppET-1 mRNA increased, peaking at 24 hours, then gradually returned to the control levels by 4 days. ppET-1 mRNA in LV was unchanged in sham-operated rats.

Plasma immunoreactive ET-1 levels increased after aortic banding, peaked at 24 hours, and then returned to the basal level at 4 days (Fig 5); plasma ET-1 did not show any significant change in sham-operated rats during 2-week periods.

Discussion

The present study demonstrated for the first time that cardiac hypertrophy with concomitant expression of skeletal α-actin and ANP gene provoked by pressure overload was partially blocked by the ET_A receptor antagonist BQ123 in vivo and that transient increases of both LV ppET-1 mRNA and plasma ET-1 levels were induced during the early phase of pressure overload.

We[10] and other investigators[11] demonstrated that ET-1 induces hypertrophy of cultured rat cardiomyo-

Table 2. Diameter of Cardiomyocytes in Experiment 1

Time of Death	Operation and Treatment	n	Cell Diameter, μm
1 wk	Sham+saline	5	14.78±0.16
	AOB+saline	5	16.64±0.48*
	Sham+BQ123	5	14.76±0.76
	AOB+BQ123	5	15.32±0.48†
2 wk	Sham+saline	5	15.38±0.33
	AOB+saline	5	17.60±0.66*
	Sham+BQ123	5	15.82±0.45
	AOB+BQ123	5	17.40±0.70*

n indicates number of rats; AOB, aortic banding. Values are mean±SEM.
*P<.05 compared with sham-operated group; †P<.05 compared with AOB+saline group.

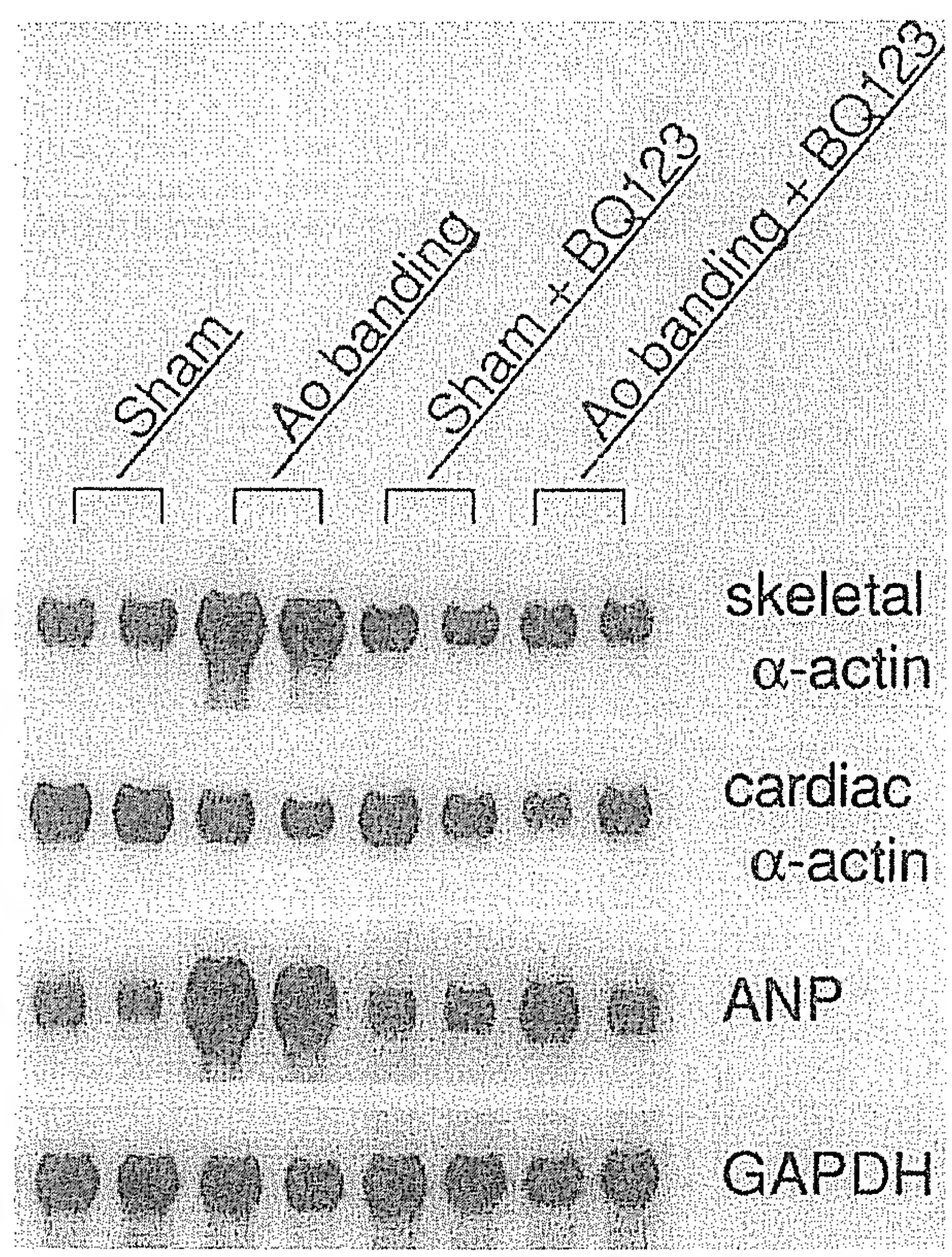

Fig 1. Representative Northern blot showing mRNA levels of skeletal and cardiac α-actin and atrial natriuretic peptides (ANP) in the left ventricle of rats after aortic (Ao) banding or sham operation with or without BQ123. Suprarenal aortic banding or sham operation was performed as described in the text. BQ123 or saline was continuously administered by an osmotic pump starting 24 hours before the operation. At 1 week after operation, rats were killed and total RNA was extracted from the left ventricular free wall. Hybridizations by Northern blot (10 μg of total RNA per lane) were performed using ^{32}P-labeled rat skeletal and cardiac α-actin, atrial natriuretic peptides, and GAPDH cDNA probes, respectively. Exposure time was 24 hours.

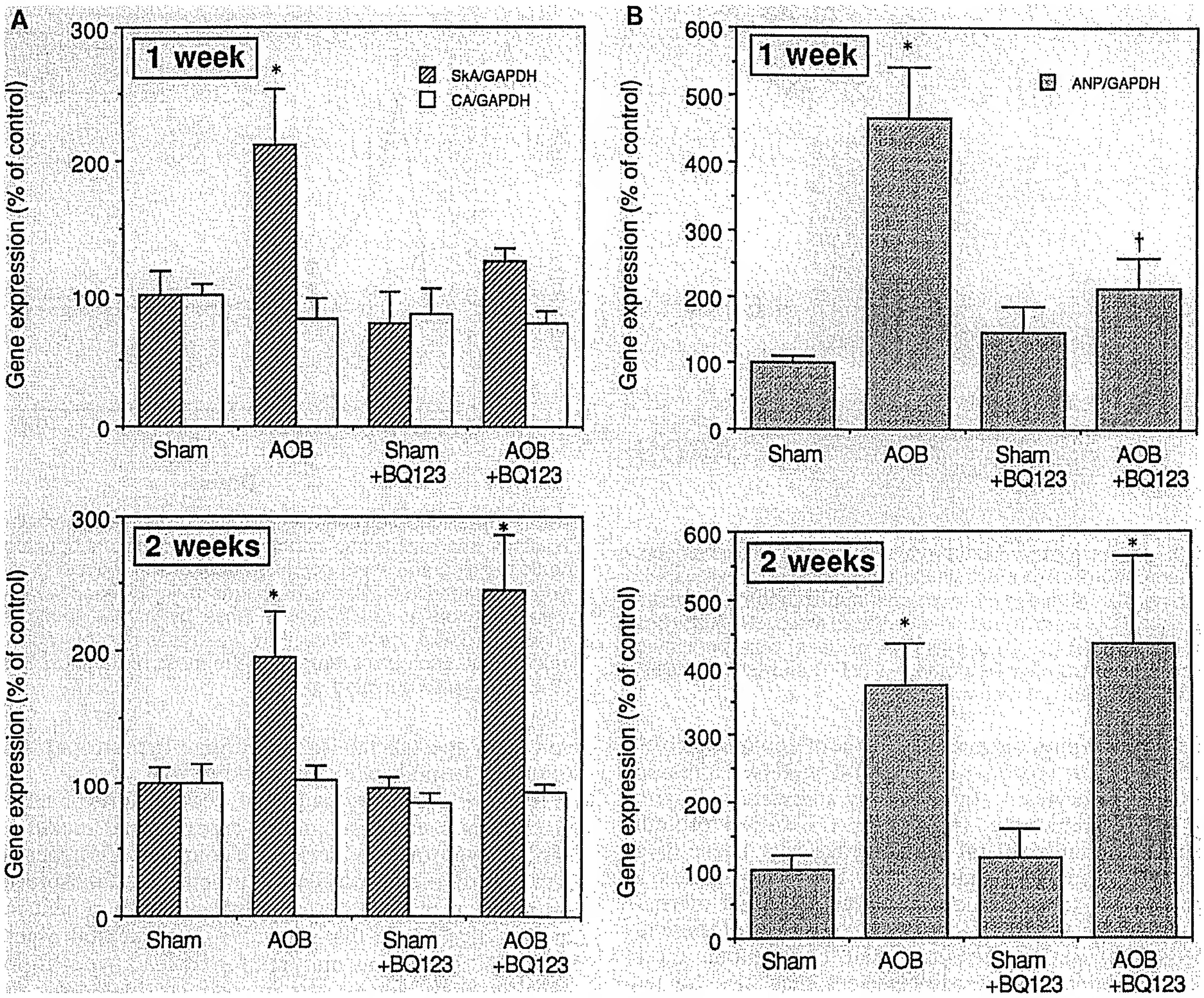

FIG 2. Bar graphs showing quantitative mRNA levels of skeletal and cardiac α-actin and atrial natriuretic peptides (ANP) in the left ventricle of rats after aortic banding or sham operation with or without BQ123. Aortic banding (AOB) and sham operation with or without BQ123 administration were performed in the same manner as in Fig 1. At 1 and 2 weeks after operation, rats were killed and total RNA was extracted from the left ventricular free wall. mRNA levels by dot blot analysis hybridized with ^{32}P-labeled probes were quantified by an image analyzer system. A. Quantitative mRNA levels of skeletal and cardiac α-actin (SkA and CA) normalized for GAPDH expression. B. Quantitative mRNA levels of ANP normalized for GAPDH expression. Data are expressed as percentage of mRNA levels compared with those of the sham-operated group. Bars show SEM. n=5 per group. *P<.05 vs sham-operated group. †P<.05 vs AOB group.

cytes with concomitant transcriptional activation of several muscle-specific genes and the c-*fos* proto-oncogene, suggesting the possible involvement of ET-1 in the development of cardiac hypertrophy. Recently, development of a novel selective ET_A receptor antagonist (BQ123)[13] has proved to be a useful tool for under-

TABLE 3. Aortic Systolic Pressure, Body Weight, and Left Ventricular Weight at Death in Experiment 2

Time of Death	Operation	n	Aortic Systolic Pressure, mm Hg	Body Weight, g	LV Weight, mg	LV Weight, mg/Body Weight, g
Control		4	117±12	272±2.3	617±10.3	2.26±0.05
6 h	Sham	5	124±15	278±4.9	628±7.0	2.23±0.01
	AOB	5	130±10	298±4.0	626±17.3	2.25±0.04
1 d	Sham	5	121±13	279±3.3	683±10.5	2.45±0.05
	AOB	5	163±14*	280±3.3	693±28.3	2.48±0.12
2 d	Sham	5	132±17	282±3.6	689±16.4	2.47±0.06
	AOB	5	168±10*	282±3.2	725±33.1	2.56±0.10
4 d	Sham	5	114±13	285±3.3	681±11.5	2.37±0.06
	AOB	4	158±18*	288±1.0	756±21.6*	2.62±0.08*

n indicates number of rats; LV, left ventricle; and AOB, aortic banding. Values are mean±SEM.
*P<.05 compared with sham-operated group.

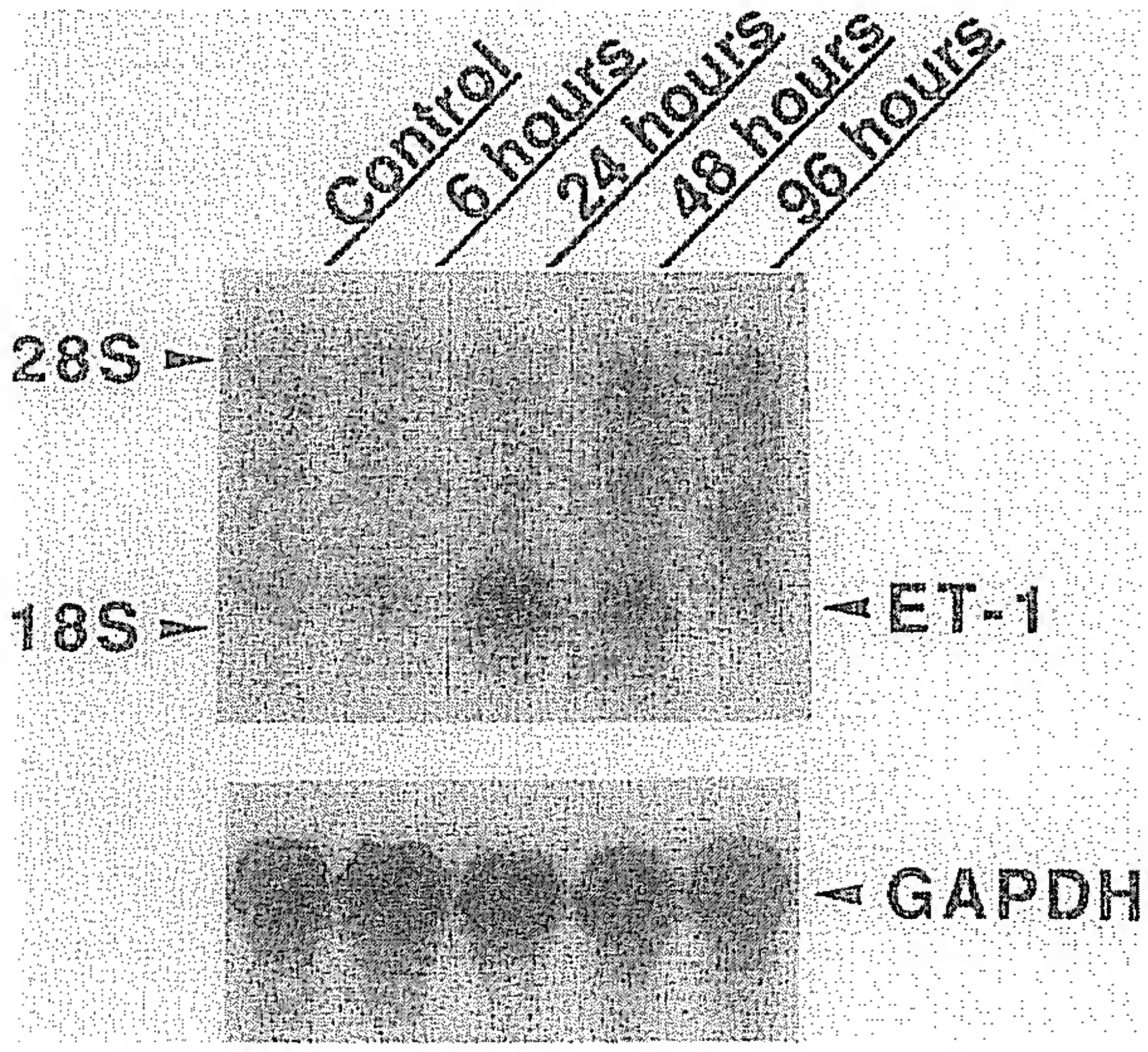

Fig 3. Representative Northern blot assessing relative amount of preproendothelin-1 mRNA in the left ventricle of rats with aortic banding. Rats with aortic banding performed in the same manner as in Fig 1 were killed at the indicated times. Hybridizations by Northern blot (20 μg of total RNA per lane) were performed using ^{32}P-labeled preproendothelin-1 (ET-1) and GAPDH cDNA as probes. Exposure time was 5 days.

standing the physiological and pathophysiological roles of ETs. In our recent study, ppET-1 mRNA expressed by cardiomyocytes is stimulated by angiotensin II, and BQ123 inhibited the angiotensin II–induced protein synthesis in cultured rat cardiomyocytes.[12] These data led us to speculate that cardiac ET-1 may act as a hypertrophy factor via an autocrine/paracrine mechanism through the ET_A receptor. On the basis of these in vitro data, we hypothesized that endogenous ET-1 locally produced by cardiomyocytes may also be involved in the mechanism of cardiac hypertrophy provoked by hemodynamic overload in vivo.

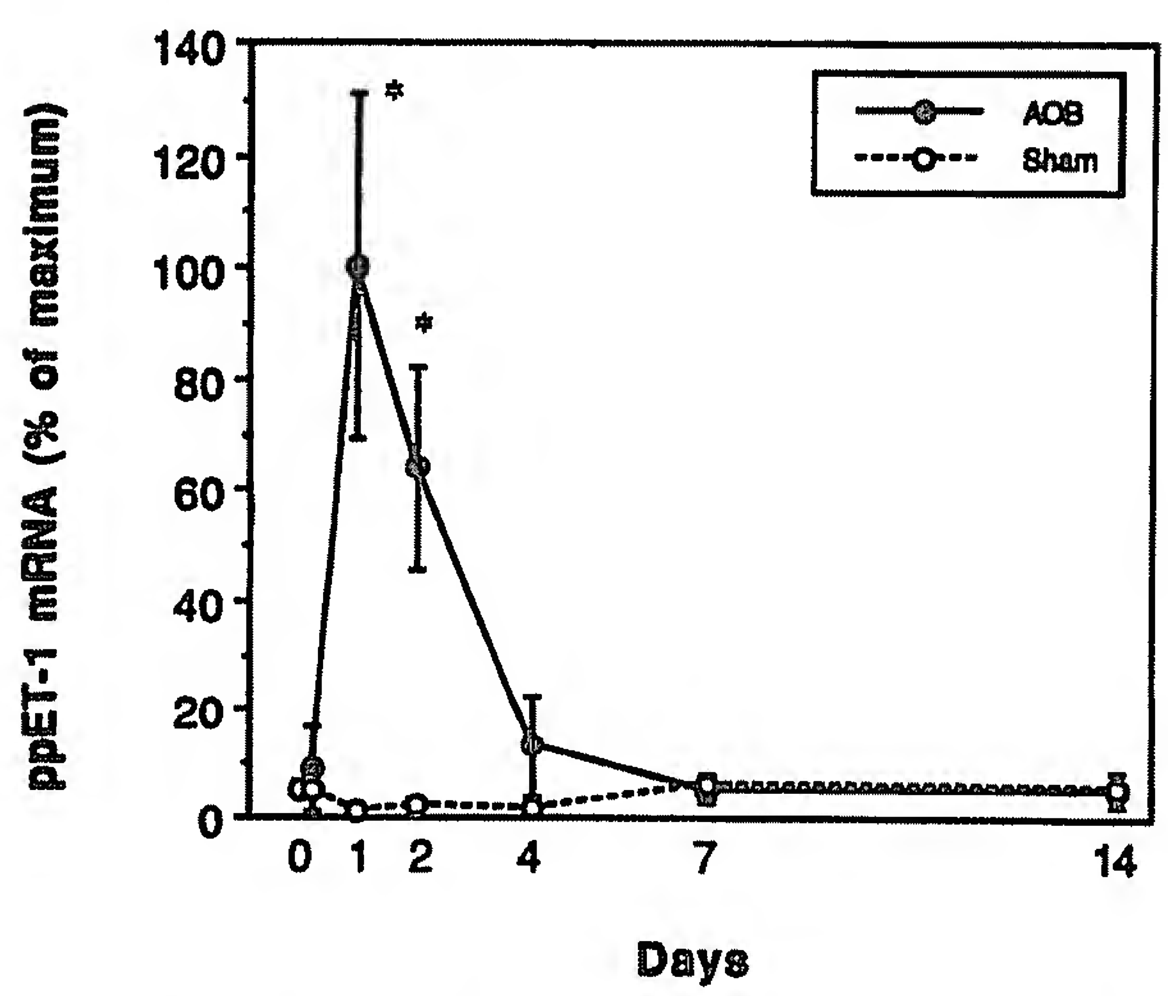

Fig 4. Graph showing quantitative mRNA levels of preproendothelin-1 (ppET-1) in the left ventricle of rats after aortic banding and sham operation. Rats with aortic banding (AOB) and sham operation performed in the same manner as in Fig 1 were killed at the indicated times (four or five rats per group). mRNA levels of preproendothelin-1 in the left ventricle assayed by dot blot analysis (10 μg of total RNA per dot) were quantified by an image analyzer system and normalized for GAPDH expression. Data are expressed as percentage of mRNA levels compared with those of control (time 0) group. Bars show SEM. *P<.05 vs control.

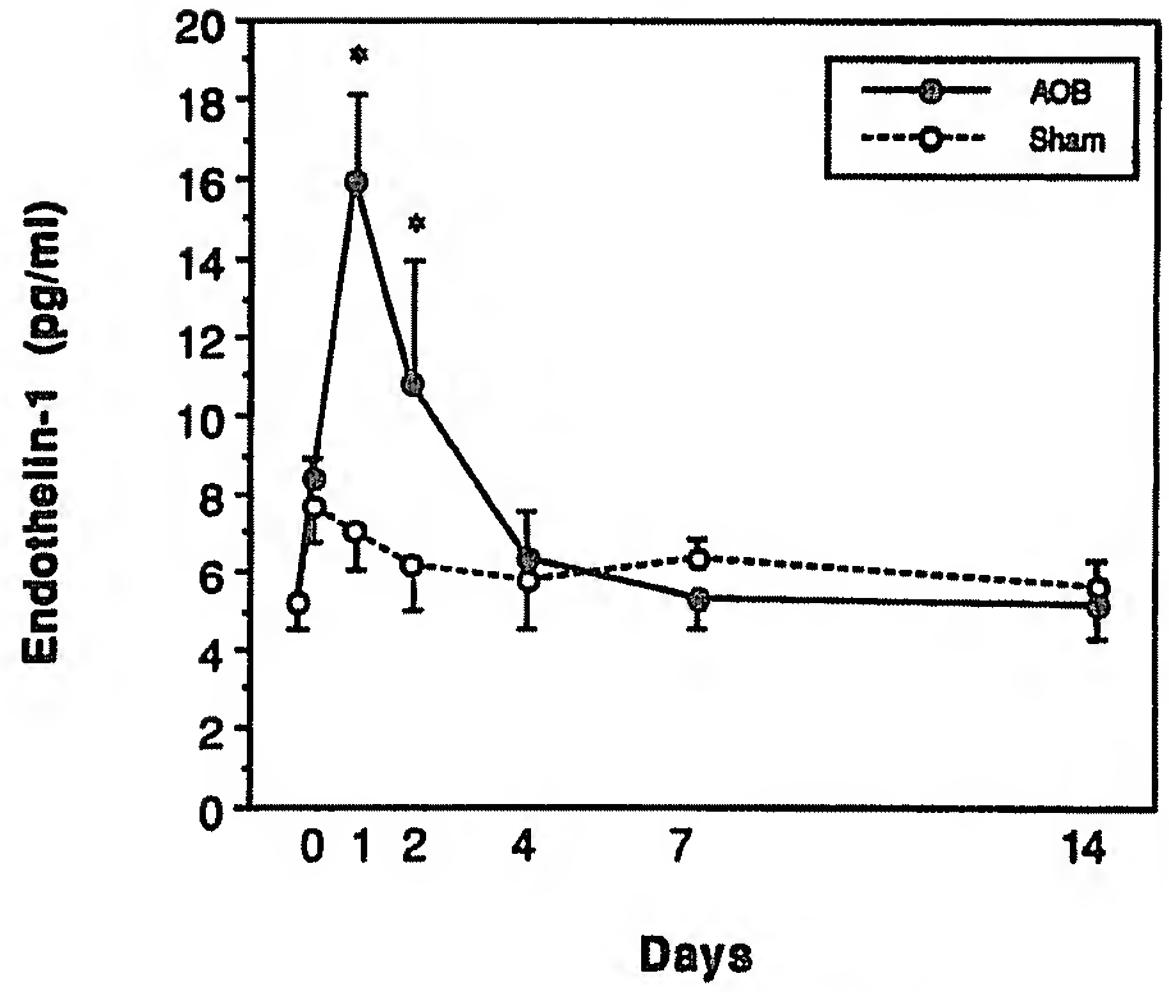

Fig 5. Graph showing changes of immunoreactive endothelin-1 in rat plasma after aortic banding (AOB) or sham operation. Aortic banding and sham operation (four or five rats per group) were performed in the same manner as in Fig 1. Blood samples were collected at the indicated times by cardiac puncture; endothelin-1–like immunoreactivity was measured by radioimmunoassay. Each point represents the mean; bars show SEM. *P<.05 vs sham-operated group.

Rats with LV overload produced by aortic banding are widely used as an in vivo experimental model for cardiac hypertrophy. During the course of LV hypertrophy, several genes encoding contractile protein isoforms ordinarily expressed in the embryonic or fetal ventricle are reactivated. In this study, we used skeletal α-actin and ANP mRNA as markers for cardiac hypertrophy, because these genes are in vivo correlates for the changes seen with hypertrophy stimulated by neurohumoral factors in vitro. The present study clearly shows the blockade actions by BQ123 on skeletal α-actin and ANP mRNA levels in the rats with aortic banding at 1 week. Taken together with the present data that BQ123 inhibited the ratio of LV weight to body weight and diameter of cardiomyocytes in the LV, our results are in accord with the hypothesis that endogenous ET-1 may play a role in the mechanism of cardiac hypertrophy provoked by hemodynamic overload. The specificity of BQ123 on ET_A receptor antagonistic action has been well examined in vivo[24] as well as in vitro[25]; therefore, it is unlikely that the inhibitory action of BQ123 on cardiac hypertrophy may be mediated by other neurohumoral factors, such as α_1-adrenergic stimulator and angiotensin II. Since BQ123 did not affect aortic pressure in rats with aortic banding or sham operation, the inhibitory effect of BQ123 on cardiac hypertrophy was not due to its direct action on blood pressure. In the present study, we also demonstrated that both ppET-1 mRNA in LV and plasma ET-1 levels were transiently increased after aortic banding, suggesting the local production of ET-1 in the cardiovascular system during the early course of cardiac hypertrophy.

Several neurohumoral and growth factors, such as α_1-adrenergic agonists,[2,3] angiotensin II,[4] basic fibroblast growth factor,[5] and insulin-like growth factor-I,[6] have been reported to induce cardiomyocyte hypertrophy in vitro. However, the contribution of such factors,

except for angiotensin II,[26] to cardiac hypertrophy induced by mechanical overload in vivo has not been proved. Thus, our study is the first report that clearly shows the causal relation of endogenous ET-1 to pressure-induced cardiac hypertrophy in vivo. The continuous infusion of BQ123 (250 μg/h) used in this study maintains its plasma levels at almost 5×10^{-7} mol/L; these concentrations were comparable to those that inhibit the cardiac hypertrophy action by angiotensin II in vitro.[12]

It should be noted that blockade effects by BQ123 on cardiac hypertrophy and ventricular skeletal α-actin and ANP gene expression were no longer observed at 2 weeks after aortic banding. Since ET_A receptor and its mRNA are not upregulated during cardiomyocyte hypertrophy in vitro,[27] it is unlikely that the inability of BQ123 to maintain an inhibitory effect is due to the compensatory upregulation of ET_A receptor on cardiomyocytes. Therefore, it is possible to speculate that cardiac ET-1 may act as an "initiating" hypertrophy factor during the early phase of pressure overload but that other factors, such as local renin-angiotensin systems and several growth factors, might take over as a "maintaining" factor during the late phase of pressure overload.

The clinical application of ET_A receptor antagonists to prevention and treatment of cardiac hypertrophy might be limited because of its short half-life in circulation and its route of administration. Nevertheless, our present results provide a possible therapeutic approach using ET receptor antagonist for cardiac hypertrophy by blockade of endogenous ET-1.

Acknowledgments

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References

1. Izumo S, Nadal-Ginard B, Mahadavi V. Protooncogene induction and reprogramming of cardiac gene expression produced by pressure overload. *Proc Natl Acad Sci U S A.* 1988;85:339-343.
2. Lee HR, Henderson SA, Reynolds R, Dunnmon P, Yuan D, Chien KR. α_1-Adrenergic stimulation of cardiac gene transcription in neonatal rat myocardial cells: effects on myosin light chain-2 gene expression. *J Biol Chem.* 1988;163:7352-7358.
3. Bishopric NH, Simpson PC, Ordahl CP. Induction of the skeletal α-actin gene in α_1-adrenoceptor-mediated hypertrophy of rat cardiac myocytes. *J Clin Invest.* 1987;80:1194-1199.
4. Katoh Y, Komuro I, Shibasaki Y, Yamaguchi H, Yazaki Y. Angiotensin II induces hypertrophy and oncogene expression in cultured rat heart myocytes. *Circulation.* 1989;80(suppl II):II-450. Abstract.
5. Parker TG, Chow KL, Schwarz RJ, Schneider MD. Differential regulation of skeletal α-actin transcription in cardiac muscle by two fibroblast growth factors. *Proc Natl Acad Sci U S A.* 1990;87:7066-7070.
6. Ito H, Hiroe M, Hirata Y, Tsujino M, Adachi S, Shichiri M, Koike A, Nogami A, Marumo F. Insulinlike growth factor–I induces hypertrophy with enhanced expression of muscle specific genes in cultured rat cardiomyocytes. *Circulation.* 1993;87:1715-1721.
7. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature.* 1988;332:411-415.
8. Hirata Y, Takagi Y, Fukuda Y, Marumo F. Endothelin is a potent mitogen for rat vascular smooth muscle cells. *Atherosclerosis.* 1989;78:225-228.
9. Takuwa N, Takuwa Y, Yanagisawa M, Yamashita K, Masaki T. A novel vasoactive peptide endothelin stimulates mitogenesis through inositol lipid turnover in Swiss 3T3 fibroblasts. *J Biol Chem.* 1989;264:7856-7861.
10. Ito H, Hirata Y, Hiroe M, Tsujino M, Adachi S, Takamoto T, Nitta M, Taniguchi K, Marumo F. Endothelin-1 induces hypertrophy with enhanced expression of muscle-specific genes in cultured neonatal rat cardiomyocytes. *Circ Res.* 1991;69:209-215.
11. Shubeita HE, McDonough PM, Harris AN, Knowlton KU, Glembotski CC, Brown JH, Chien KR. Endothelin induction of inositol phospholipid hydrolysis, sarcomere assembly, and cardiac gene expression in ventricular myocytes: a paracrine mechanism for myocardial cell hypertrophy. *J Biol Chem.* 1990;265:20555-20562.
12. Ito H, Hirata Y, Adachi S, Tanaka M, Tsujino M, Koike A, Nogami A, Marumo F, Hiroe M. Endothelin-1 is an autocrine/paracrine factor in the mechanism of angiotensin II-induced hypertrophy in cultured rat cardiomyocytes. *J Clin Invest.* 1993;92:398-403.
13. Ihara M, Noguchi K, Saeki T, Fukuroda T, Tsuchida S, Kimura S, Fukami T, Ishikawa K, Nishikabe M, Yano M. Biological profiles of highly potent novel endothelin antagonists selective for the ET_A receptor. *Life Sci.* 1992;50:247-255.
14. Maki M, Takayanagi R, Misono K, Pandy KN, Tibbitts C, Iangami T. Structure of rat atrial natriuretic factor precursor deduced from cDNA sequence. *Nature.* 1984;21:723-725.
15. Oikawa S, Imai M, Ueno A, Tanaka S, Noguchi T, Nakazato H, Kangawa K, Fukuda A, Matsuo H. Cloning and sequence analysis of cDNA encoding a precursor for human atrial natriuretic polypeptide. *Nature.* 1984;309:724-726.
16. Webster KA. Regulation of enzyme RNA transcriptional rates by oxygen availability in skeletal muscle cells. *Mol Cell Biochem.* 1987;77:19-28.
17. Jouannot P, Hatt PY. Rat myocardial mechanics during pressure-induced hypertrophy development and reversal. *Am J Physiol.* 1975;229:355-364.
18. Hiroe M, Ohta Y, Fujita N, Nagata M, Toyoda T, Kusakage K, Sekiguchi M, Marumo F. Myocardial uptake of ^{111}In monoclonal antimyosin Fab in detecting doxorubicin cardiotoxicity in rats. *Circulation.* 1992;86:1965-1972.
19. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 1979;18:5294-5299.
20. Feinberg AP, Vogelstein BA. Technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem.* 1983;132:6-13.
21. Ito H, Miller SC, Billingham ME, Akimoto H, Torti S, Wade R, Gahlmann R, Lyons G, Kedes L, Torti FM. Doxorubicin selectively inhibits muscle gene expression in cardiac muscle cells in vitro and in vivo. *Proc Natl Acad Sci U S A.* 1990;87:4275-4279.
22. Ando K, Hirata Y, Takei Y, Kawakami M, Marumo F. Endothelin-1-like immunoreactivity in human urine. *Nephron.* 1991;57:36-39.
23. Ohta K, Hirata Y, Shichiri M, Kanno K, Emori T, Tomita K, Marumo F. Urinary excretion of endothelin-1 in normal subjects and patients with renal disease. *Kidney Int.* 1991;39:307-311.
24. McMurdo L, Corder R, Thiemermann C, Vane JR. Incomplete inhibition of the pressor effects of endothelin-1 and related peptides in the anaesthetized rat with BQ123 provides evidence for more than one vasoconstrictor receptor. *Br J Pharmacol.* 1993;108:557-561.
25. Ihara M, Noguchi K, Saeki T, Fukuroda T, Kimura S, Fukami T, Ishikawa K, Nishikibe M, Yano M. Biological profiles of highly potent novel endothelin antagonists selective for the ET_A receptor. *Life Sci.* 1992;50:247-255.
26. Baker KM, Chernin MI, Wixson SK, Aceto JF. Renin-angiotensin system involvement in pressure-overload cardiac hypertrophy in rats. *Am J Physiol.* 1990;259:H324-H332.
27. Kanno K, Hirata Y, Tsujino M, Imai T, Shichiri M, Ito H, Marumo F. Upregulation of ET_B receptor subtype mRNA by angiotensin II in rat cardiomyocytes. *Biochem Biophys Res Commun.* 1993;194:1282-1287.